INDUCTION OF APOPTOSIS IN CATECHOLAMINERGIC PC12 CELLS BY L-DOPA - IMPLICATIONS FOR THE TREATMENT OF PARKINSON'S DISEASE.

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Levodopa (L-DOPA), the natural precursor to dopamine, is the most effective and frequently prescribed drug for the symptomatic treatment of Parkinson's disease. However, conflicting reports have suggested that L-DOPA therapy may or may not augment neuronal damage and thus accelerate the progression of the disease. In this study we have investigated the toxic effects of L-DOPA on catecholaminergic PC12 cells *in vitro*, and examined the mechanism underlying L-DOPA toxicity.

Treatment of PC12 cells with clinically applicable concentrations of L-DOPA (25-100μM) induced cell death via a mechanism which exhibited morphological and biochemical characteristics of apoptosis, including chromatin condensation, membrane blebbing, and internucleosomal DNA fragmentation. Induction of apoptosis by L-DOPA was specific to catecholaminergic cells and not a general phenomenon observed in all cell types, since no cell death was observed following treatment of rat hepatoma FaO cells or rat-1 fibroblasts with 50μM L-DOPA. Treatment of PC12 cells with 25μM carbidopa, which inhibits the conversion of L-DOPA to dopamine, did not suppress apoptosis induced by 50μM L-DOPA. Thus, toxicity appeared to be a direct effect of L-DOPA itself, rather than mediated via its conversion to dopamine. Induction of apoptosis by 50μM L-DOPA was inhibited by addition of antioxidants such as glutathione (1mM), ascorbic acid (100μM), or vitamin E (100μM), suggesting that activation of apoptosis by L-DOPA was mediated by generation of reactive oxygen species. Induction of apoptotic cell death by L-DOPA was preceded by a rapid transient increase in expression of the early response genes *c-fos* and *egr-1*, within 30-45 minutes of addition of the drug. The role that these, and other early response genes, play in the induction of apoptosis by neurotoxins such as L-DOPA and 6-hydroxydopamine, is under investigation.

Our findings demonstrate that a drug used to treat the symptoms of Parkinson's disease induces apoptosis of catecholaminergic cells *in vitro*. Since the toxicity of L-DOPA appears to be mediated via an active cellular programme of apoptosis rather than passive necrosis, therapeutic approaches may be devised to decrease L-DOPA toxicity *in vivo* and thus prevent acceleration of neuronal damage in Parkinson's disease.